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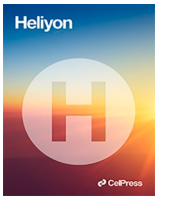

Research article

# Combined serum CTRP7 and CTRP15 levels as a novel biomarker for insulin resistance and type 2 diabetes mellitus

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ABSTRACT

*Aims:* This study aimed to examine the alterations in the serum CTRP7 and CTRP15 concentrations in patients newly diagnosed with type 2 diabetes mellitus (T2DM) and to assess the diagnostic potential of the log10 (CTRP7+CTRP15) for insulin resistance (IR) and T2DM.
*Methods:* Serum CTRP7, CTRP15, and adiponectin levels were measured using an enzyme-linked immunosorbent assay (ELISA). Bioinformatics analysis was conducted to investigate CTRP7 and CTRP15-related genes and metabolic signaling pathways.
*Results:* Log10 (CTRP7+CTRP15) levels were notably elevated in the impaired glucose tolerance (IGT) and T2DM cohorts compared with those in the normal control (NGT) cohort. Log10 (CTRP7+CTRP15) exhibited positive correlations with HOMA-IR, area under the glucose curve (AUCg), HbA1c%, triglyceride (TG), visceral adiposity index (VAI), body mass index (BMI), and free fatty acid (FFA), levels but negative correlations with adiponectin. Multivariate stepwise regression analysis revealed that HOMA-IR, BMI, HbA1c and FFA levels were independent factors affecting the log10 (CTRP7+CTRP15). Logistic regression analysis revealed that log10 (CTRP7+CTRP15) was independently associated with T2DM and significantly associated with increased risk. Receiver operating characteristic (ROC) curve analysis indicated that the predictive value of log10 (CTRP7+CTRP15) for T2DM and IR was superior to that of CTRP7 or CTRP15 alone. Intervention studies demonstrated that insulin, FFAs and acute exercise contribute to the elevation of serum CTRP7 levels, while hyperglycemia inhibited CTRP7 secretion. Short-term changes in blood glucose, insulin, FFA and acute exercise had minimal effects on serum CTRP15 levels. Bioinformatics analysis revealed that CTRP7 and CTRP15 interact with multiple metabolism-related genes and are enriched in glucose and lipid metabolism-related pathways.
*Conclusion:* Log10 (CTRP7+CTRP15) may serve as a valuable diagnostic marker for the management of metabolic-related diseases, particularly T2DM and IR.

## 1. Introduction

Adipose tissue, the largest endocrine organ, secretes a multitude of adipokines that regulate glucose and lipid metabolism and

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participate in various physiological and pathological processes, such as inflammation, apoptosis, autoimmunity, and insulin resistance (IR). An imbalance between proinflammatory and protective adipokines in vivo can result in IR and metabolic disturbances [1,2]. Thus, investigating the role and molecular mechanisms of adipocyte-derived factors in metabolic diseases holds great significance in discovering novel therapeutic targets for antidiabetic drugs.

The C1q complement/TNF-related protein family (CTRP) is a newly discovered adipokine family consisting of 15 members (CTRP1-15), all of which share a similar structure with adiponectin (APN) [3,4]. CTRPs are implicated in insulin signaling, energy metabolism, and vascular inflammation [5]. CTRP7 is a highly conserved secretory protein found in vertebrates and is a secreted regulator of inflammation, cellular stress, insulin sensitivity, and glucose metabolism [6]. Previous studies have shown elevated CTRP7 levels in adipose and hepatic tissues of obese (ob/ob) and diabetic (db/db) mice, while CTRP7 deletion improved glucose metabolism and IR in high-fat diet (HFD)-fed mice [6,7]. Similarly, compared with those in healthy controls, serum CTRP7 levels were found to be elevated in patients with obesity, T2DM and metabolic syndrome (MetS) and closely correlated with blood glucose levels, free fatty acids (FFAs), and HOMA-IR [7,8].

CTRP15, also known as myonectin, is primarily derived from skeletal muscle and coordinates the integration of whole-body metabolism [9]. CTRP15 enhances reverse cholesterol transport efficiency and increases plasma HDL-C levels via the T-cadherin/miR-101-3p/ABCA1 pathway [10]. Increased CTRP15 expression has been observed in the adipose tissue of obese mice, while aerobic exercise has been reported to elevate circulating CTRP15 levels [11]. Our cross-sectional study revealed significantly higher serum CTRP15 levels in persons with T2DM and MetS than in healthy individuals, exhibiting a positive correlation with obesity-related indicators, insulin levels, and proinflammatory cytokine levels [12,13]. However, other studies have shown that reduced serum CTRP15 levels are associated with obesity, diabetes, and polycystic ovary syndrome (PCOS) and are negatively correlated with IR [14,15]. Therefore, the precise role of CTRP15 in IR and metabolic disorders remains to be elucidated.

Prior studies suggest that CTRP7 and CTRP15 may serve as potential serum biomarkers for metabolic diseases. However, to date, no studies have reported on potential synergistic or antagonistic interactions between CTRP7 and CTRP15, and it is unclear whether changes in metabolic status have combined effects on CTRP7 and CTRP15. In this study, we established a novel combined index of serum CTRP7 and CTRP15, log10 (CTRP7+CTRP15), for the first time. We compared the alterations of these genes across healthy individuals, persons with prediabetes, and persons with newly diagnosed T2DM to determine the relationships of these genes with glucose and lipid metabolism-related indicators. Additionally, we evaluated the predictive value of the log10 (CTRP7+CTRP15) for IR and investigated the coordinated changes and potential regulatory mechanisms of CTRP7 and CTRP15 in different metabolic states through intervention studies.

## 2. Materials and methods

### 2.1. Study population

The present investigation included a total of 335 participants (166 males and 169 females, with an average age of 54 years), comprising 112 persons with newly diagnosed T2DM, 108 persons with impaired glucose tolerance (IGT), and 115 normal glucose tolerance (NGT) controls. Participants were recruited from healthy individuals undergoing physical examinations or patients from endocrinology departments of the Second Affiliated Hospital of Chongqing Medical University. Diagnostic assessments of T2DM, IGT, and NGT were performed based on the established guidelines of the American Diabetes Association (ADA) using the 75 g oral glucose tolerance test (OGTT) [16]. All persons with T2DM and IGT were newly diagnosed, with no prior pharmacological or lifestyle interventions reported. Patients with acute or chronic complications, such as diabetic ketoacidosis (DKA); hypertension; and significant organ diseases, such as cardiac, hepatic, and renal disorders, were excluded. The selection parameters for the inclusion of healthy control subjects adhered to previous guidelines [12]. The experimental design is shown in Fig. 1. Informed consent was obtained from all participants prior to their engagement in the research process. This study was approved by the Human Research Ethics Committee of Chongqing Medical University (2012 Ethical Review No.74) and registered at the Chinese Clinical Trial Registry (ChiCTR–OCS–13003185). All participants provided informed consent to participate in the study.

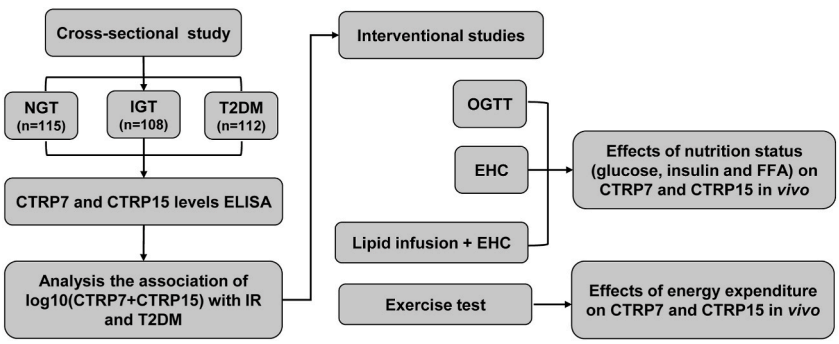


**Fig. 1.** Clinical experimental design. OGTT, oral glucose tolerance test; EHC, euglycemic-hyperinsulinemic clamp.

### 2.2. Assessment of anthropometric and biochemical parameters

After an overnight fast of 10–12 h, physical examinations were performed by professional physicians, and venous blood samples were collected from all participants in this study for analytical purposes. The methods for conducting the physical examination and assessing biochemical parameters were implemented as previously described [17]. The homeostasis model assessment of IR (HOMA-IR) was calculated as previously described. A HOMA-IR $\geq$ 3.0 was considered indicative of IR [18].

### 2.3. Serum CTRP7, CTRP15 and APN measurements

The serum concentrations of CTRP7 and CTRP15 were determined using ELISA kits (sk00396-09 and sk00393-19, Aviscera Bioscience Inc., USA) according to the manufacturer's instructions. In CTRP7 assessment, both intra- and inter-assay variations were meticulously contained, demonstrating coefficients of variation (CV) less than 5 % and 10 %, respectively. For CTRP15 evaluation, the corresponding intra- and inter-assay CVs were below 8 % and 12 %, respectively. APN serum concentrations were determined using an ELISA kit (sk00010-01, Aviscera Bioscience Inc., USA) with intra- and inter-assay CVs of 4–8% and 8–12 %, respectively. The composite index, log10 (CTRP7+CTRP15), was calculated based on the serum concentrations of CTRP7 and CTRP15.

### 2.4. OGTT and hyperinsulinemic-euglycemic clamp (EHC)

Following an overnight fast of 10–12 h, all participants underwent an OGTT as previously reported [12]. We procured blood samples at specific intervals—at baseline and at 30, 60, and 120 min—for the quantification of glucose and insulin concentrations. Serum levels of CTRP7 and CTRP15 were assessed at the corresponding time points.

We conducted EHC experiments in 24 healthy young individuals (12 males and 12 females, mean age 25.3 years) as previously reported [17]. During the course of EHC, blood samples were specifically obtained at time intervals of 0, 80, 100, and 120 min. Subsequently, serum was methodically isolated via centrifugation and preserved at −80 °C for subsequent analyses.

### 2.5. Lipid infusion study

A total of 19 healthy participants (10 males and 9 females, mean body mass index (BMI) 20.9 $\pm$ 2.5 kg/m$^2$) participated in the lipid infusion experiment. The lipid infusion study was conducted as previously reported [12]. These subjects received a 20 % intralipid/heparin (0.4 units/kg/min; Pharmacia and Upjohn, Milan, Italy) intravenous infusion at a constant rate (1.5 mL/min) for 240 min. The EHC experiment was initiated 2 h after the start of lipid infusion. Blood samples were acquired at predetermined time intervals before lipid infusion and during the EHC and stored at −80 °C for subsequent analyses.

### 2.6. Exercise experiment

A total of 16 healthy participants (8 males and 8 females) participated in a short-term exercise experiment. Briefly, following overnight fasting, participants performed a 45-min run at 60 % VO2 max (ParvoMedics Metabolic Measurement System, ParvoMedics) between 8:00 and 8:45 a.m. the following day. We obtained blood samples at four time intervals: baseline and 45, 60, and 120 min postexercise. Individuals with contraindications to physical activity and those who engaged in at least 20 min of exercise twice a week were excluded.

### 2.7. Bioinformatics analysis

#### 2.7.1. Construction of the protein–protein interaction (PPI) network

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (version 11.0; http://string-db.org/) was used to analyse the interactions between CTRP7, CTRP15, and other proteins. In the PPI network diagram, an interaction score exceeding 0.4 was designated as the threshold criterion [19].

#### 2.7.2. Gene function enrichment analysis

REACTOME pathway enrichment analysis was conducted using the STRING database, and an FDR <0.01 was considered to indicate statistical significance for identifying genes related to glucose/lipid metabolism for further analysis [20]. The R software clusterProfiler package (Clusterprofiler r package) was used for GO and KEGG enrichment analysis [21]. After the gene list was uploaded, the results of biological process (BP), molecular function (MF), cellular component (CC), and KEGG pathway enrichment analysis were obtained, and $p < 0.05$ was considered to indicate statistical significance.

### 2.8. Statistical analysis

All the statistical analyses were performed using SPSS software (version 26.0; Chicago, IL). The data are presented as the mean $\pm$ SD or median (interquartile range (25 %–75 %)). To achieve a normal distribution, variables that initially exhibited a nonnormal distribution were log-transformed. Group comparisons were made using analysis of variance (ANOVA), and post hoc pairwise comparisons were conducted using the least significance difference (LSD) test. Relationships between log10 (CTRP7+CTRP15) and other

variables were assessed through simple correlation or analysis while controlling for covariates. Independent correlates of log10 (CTRP7+CTRP15) were determined using multiple linear regression analysis. The associations of the log10 (CTRP7+CTRP15) with IGT and T2DM were assessed using multiple logistic regression analysis. The Cochran-Armitage trend test and row mean score analyses were used to examine the trends in log10 (CTRP7+CTRP15) values among persons with IGT and T2DM. Receiver operating characteristic (ROC) curves were constructed to evaluate the sensitivity and specificity of the log10 (CTRP7+CTRP15) model for predicting IGT and T2DM. A $p$ value $< 0.05$ was considered to indicate statistical significance compared to the control group.

## 3. Results

### 3.1. Serum CTRP7 and CTRP15 levels in populations with different glucose tolerances

Table 1 displays the clinical and biochemical characteristics of the study participants, including those with NGT, IGT, and newly diagnosed T2DM. In the IGT and T2DM groups, glucose and lipid metabolism-related indicators, including BMI, waist circumference (WC), blood pressure (BP), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), free fatty acid (FFA), HbA1c, fasting blood glucose (FBG), 2-h blood glucose after glucose overload (2 h-BG), fasting insulin (FIns), 2-h plasma insulin after glucose overload (2 h-Ins), area under the glucose curve (AUCg), HOMA-IR and visceral adiposity index (VAI), were significantly higher than those in the NGT group, while high-density lipoprotein cholesterol (HDL-C) was significantly lower. Furthermore, LDL-C, FFA, HbA1c, FBG, 2 h-BG, FIns, 2 h-Ins, AUCg, and HOMA-IR were significantly higher in the T2DM group than in the IGT group (Table 1).

The serum CTRP7 and CTRP15 levels in 90 % of healthy individuals ranged from 78.5 to 144.0 μg/L and 14.0–71.7 μg/L, respectively, while log10 (CTRP7+CTRP15) values were between 2.0 and 2.3 (Fig. 2A). Log10 (CTRP7+CTRP15) levels were significantly higher in the IGT and T2DM groups than in the NGT group, with the T2DM group showing higher levels than the IGT group (Table 1 and Fig. 2B). Additionally, the serum APN concentration was significantly lower in the persons with IGT and T2DM than in the NGT group, and it was further lower in the persons with T2DM than in the IGT group (Table 1). To further explore the relationship between log10 (CTRP7+CTRP15) and IR, we divided all participants into an IR group (HOMA-IR $\geq 3.0$, n = 106) and a non-IR group (HOMA-IR $< 3.0$, n = 229). The IR group showed significantly higher log10 (CTRP7+CTRP15) levels than the non-IR group ($7.56 \pm 0.34$ *vs.* $5.47 \pm 0.30$, $p < 0.01$) (Fig. 2C).

**Table 1**
Anthropometrics and metabolic parameters in study population.

| Variable | NGT (n = 115) | IGT (n = 108) | T2DM (n = 112) |
|---|---|---|---|
| Sex(female/mcale) | 58/57 | 56/52 | 55/57 |
| Age (years) | 53.9 ± 6.2 | 54.9 ± 10.6 | 53.6 ± 8.9 |
| BMI (kg/m$^2$) | 23.4 ± 2.3 | 24.9 ± 2.4[b] | 25.2 ± 2.9[b] |
| WC (cm) | 81.6 ± 8.4 | 86.0 ± 6.9[b] | 87.9 ± 7.4[b] |
| SBP (mmHg) | 124.0 ± 17.4 | 133.1 ± 16.8[b] | 130.0 ± 12.9[a] |
| DBP (mmHg) | 78.6 ± 11.1 | 81.1 ± 10.5 | 83.4 ± 10.2[b] |
| TC (mmol/L) | 4.70 ± 0.72 | 5.01 ± 0.88[a] | 5.21 ± 0.51[b] |
| TG (mmol/L) | 1.33 ± 0. 66 | 2.14 ± 0.86[b] | 2.11 ± 0.56[b] |
| HDL-C (mmol/L) | 1.43 ± 0.35 | 1.39 ± 0.29 | 1.19 ± 0.11[ba] |
| LDL-C (mmol/L) | 2.58 ± 0.62 | 2.6 ± 0.58 | 3.04 ± 0.21[ba] |
| FFA (μmol/L) | 0.50 ± 0.23 | 0.59 ± 0.15[b] | 0.66 ± 0.09[ba] |
| HbA1c (%) | 5.50 ± 0.50 | 5.80 ± 0.50[b] | 8.50 ± 1.00[ba] |
| FBG (mmol/L) | 5.04 ± 0.36 | 5.95 ± 0.52[b] | 7.01 ± 0.77[ba] |
| 2 h-BG (mmol/L) | 6.26 ± 1.07 | 8.32 ± 1.09[b] | 13.2 ± 1.39[ba] |
| FIns (mU/L) | 8.44 ± 1.08 | 9.75 ± 1.65[b] | 12.3 ± 3.69[ba] |
| 2 h-Ins (mU/L) | 34.5 (25.2–58.5) | 65.4 (49.9–93.5)[b] | 49.7 (33.0–69.8)[ba] |
| $AUC_g$ | 14.9 (12.9–16.5) | 19.0 (18.0–20.3)[b] | 28.3 (26.6–33.4)[ba] |
| $AUC_i$ | 84.6 (76.7–96.1) | 118.3 (82.0–158.9)[b] | 64.2 (47.1–92.1)[a] |
| HOMA-IR | 1.92 (1.70–2.09) | 2.57 (2.15–3.02)[b] | 3.81 (2.85–4.46)[ba] |
| VAI | 1.42 (0.93–1.99) | 2.25 (1.52–3.25)[b] | 2.69 (2.20–3.29)[b] |
| APN (mg/L) | 11.5 (7.1–16.8) | 7.36 (3.98–12.2)[a] | 5.04 (2.99–9.15)[ba] |
| CTRP7 (μg/L) | 112.1 (87.4–129.8) | 133.0 (109.0–176.6)[b] | 192.6 (149.9–209.5)[ba] |
| CTRP15 (μg/L) | 34.5 (25.2–58.5) | 65.5 (42.2–86.0)[b] | 77.7 (63.6–100.5)[ba] |
| log10 (CTRP7+CTRP15) | 2.17 ± 0.10 | 2.31 ± 0.12[b] | 2.41 ± 0.11[ba] |

Values are given as the mean ± SD or median (Inter quartile Range). BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density loprotein cholesterol; FFA, free fatty acid; FBG, fasting blood glucose; 2 h-BG, 2-h blood glucose after glucose overload; FIns, fasting plasma insulin; 2 h-Ins, 2-h plasma insulin after glucose overload; AUCi, the area under the curve for insulin; AUCg, the area under the curve for glucose; HOMA-IR, homeostasis model assessment of insulin resistance; VAI, visceral adiposity index; APN, adiponectin.

[a] $p < 0.05$.

[b] $p < 0.01$ compared with NGT group; $\Delta p < 0.05$.

[a] $p < 0.01$ compared with IGT group.

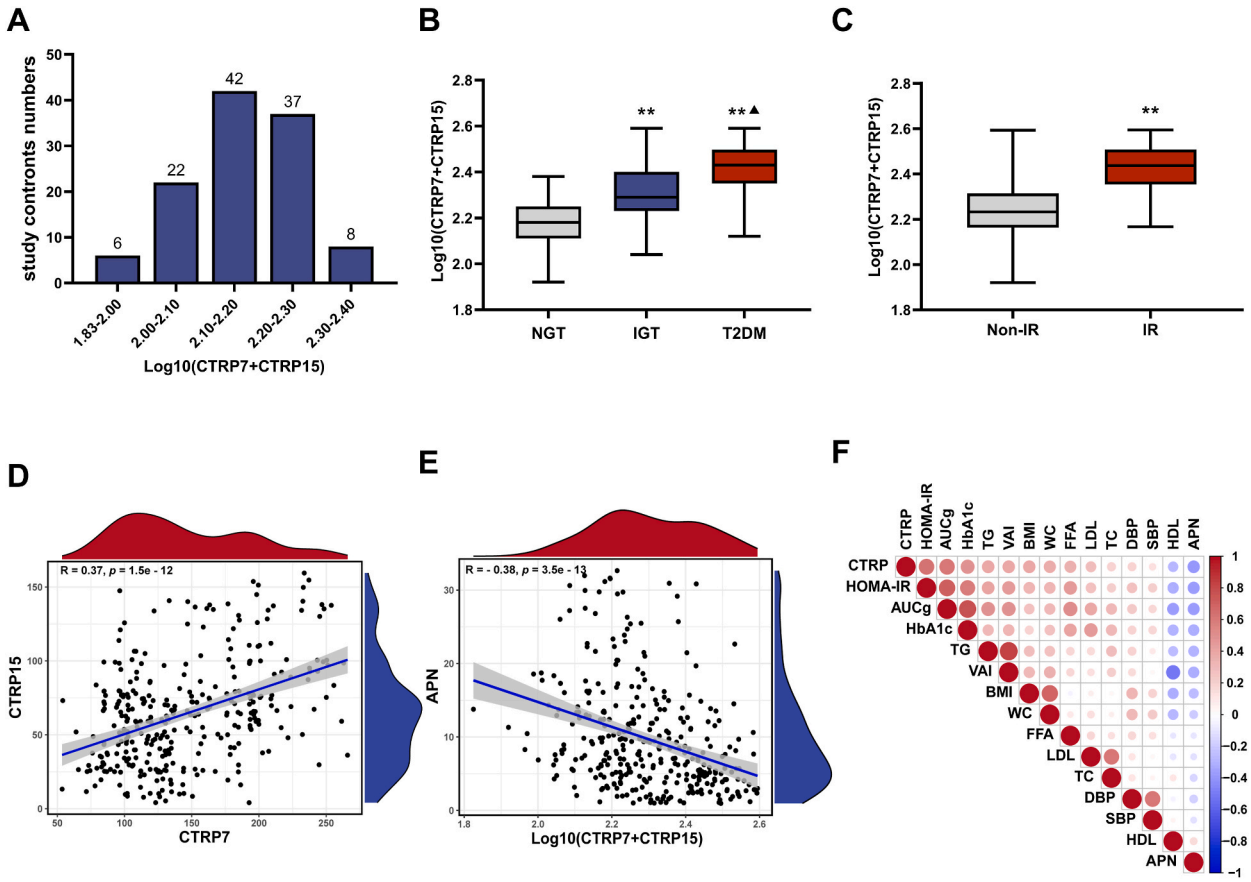


**Fig. 2.** Serum log10 (CTRP7+CTRP15) levels in the study population. **(A)** Distribution of log10 (CTRP7+CTRP15) in 115 healthy subjects. **(B)** Log10 (CTRP7+CTRP15) levels in healthy subjects, IGT and T2DM. **(C)** Log10 (CTRP7+CTRP15) levels according to HOMA-IR (IR: HOMA-IR >3; non-IR: HOMA-IR ≤ 3). **(D)** Correlations between serum CTRP7 and CTRP15. **(E)** Correlations between log10 (CTRP7+CTRP15) and adiponectin. **(F)** Correlations between log10 (CTRP7+CTRP15) and glycolipid metabolic indices. The darker the red, the stronger the correlation. CTRP, log10 (CTRP7+CTRP15); APN, adiponectin; $^{**}p < 0.01$ *vs.* NGT or controls. $^{▲}p < 0.01$ *vs.* IGT.

### *3.2. Relationships between the log10 (CTRP7+CTRP15) and clinical biochemical parameters*

Linear correlation analysis revealed that the serum CTRP7 concentration was positively correlated with the serum CTRP15 concentration in all study participants (r = 0.37, $p < 0.01$; Fig. 2D), while the log10 (CTRP7+CTRP15) was negatively correlated with the serum APN concentration (r = - 0.38, $p < 0.01$; Fig. 2E). Further analysis revealed that log10 (CTRP7+CTRP15) was positively correlated with obesity-related indicators (BMI, WC, TGs, FFAs, LDL) and IR-related indicators (HOMA-IR, AUCg, VAI, HbA1c) (all $p <$ 0.01; Fig. 2F) and negatively correlated with HDL cholesterol. Multivariate regression analysis revealed that HOMA-IR, HbA1c, APN, BMI, and FFAs were independent factors influencing the log10 (CTRP7+CTRP15) (Fig. 3A). The regression equation was $Y_{\log10\ (CTRP7+CTRP15)} = 1.695 + 0.049X_{HOMA\text{-}IR} + 0.088X_{FFA} + 0.016X_{HbA1c} + 0.014X_{BMI} - 0.003X_{APN}$ ($R^2 = 0.49$).

### *3.3. Relationships between log10 (CTRP7+CTRP15) and IGT and T2DM*

Multivariate logistic regression analysis revealed a significant association between log10(CTRP7+CTRP15) and the presence of T2DM and IR, independent of age, sex, blood pressure, BMI, WC, and serum lipids (Table 2). To further investigate the role of log10 (CTRP7+CTRP15) in body weight changes and obesity, participants were further classified into lean (BMI <24 kg/m$^2$) and overweight/obese (BMI ≥24 kg/m$^2$) groups. In the healthy population, overweight/obesity did not alter log10 (CTRP7+CTRP15) levels (Fig. 3B). Compared with healthy controls, overweight/obese individuals with IGT or T2DM exhibited elevated log10 (CTRP7+CTRP15) values (Fig. 3B).

Participants were further stratified into four categories based on log10 (CTRP7+CTRP15) levels (level 1: <2.19; level 2: 2.19–2.29; level 3: 2.29–2.41; level 4: >2.41) or serum APN levels (level 1: <4.47 mg/L; level 2: 4.47–7.75 mg/L; level 3: 7.75–13.35 mg/L; level 4: >13.35 mg/L). As shown in Fig. 3C and D, there was a notably elevated risk of T2DM and IR at level 3 and level 4 of log10 (CTRP7+CTRP15) levels for T2DM (level 3: OR, 8.41; 95 % CI, 3.29–21.51; level 4: OR, 38.38; 95 % CI, 14.60–100.91) and for IR (level 3: OR, 16.62; 95 % CI, 4.84–57.05; level 4: OR, 74.86; 95 % CI, 21.42–261.61); conversely, the risk progressively decreased as serum

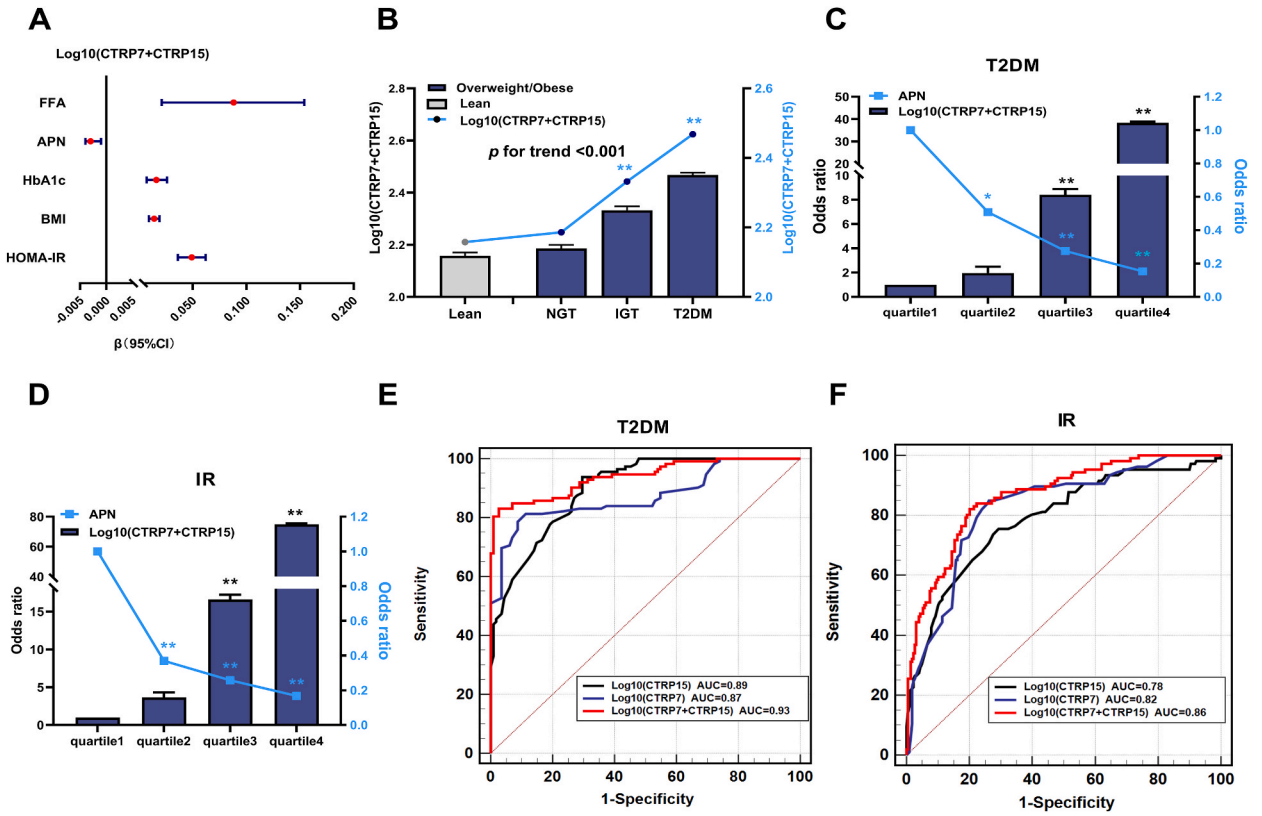


**Fig. 3.** Association of log10 (CTRP7+CTRP15) with IR and T2DM. **(A)** Stepwise multiple regression analyses of log10 (CTRP7+CTRP15) in study individuals. The circles correspond to the regression coefficients (β), and the error bars indicate the 95 % confidence interval (CI) of β. **(B)** Changes in log10 (CTRP7+CTRP15) in study individuals with different body weights and glucose metabolism levels. **(C)** Prevalence of elevated T2DM in different tertiles of log10 (CTRP7+CTRP15) and adiponectin. **(D)** Prevalence of elevated IR in different tertiles of log10 (CTRP7+CTRP15) and adiponectin. **(E)** ROC curve analysis of the prediction of T2DM. **(F)** ROC curve analysis of the prediction of IR. Data are means ± SME. $^{*}p < 0.05$ or $^{**}p < 0.01$ *vs.* Controls, lean, no IR or quartile 1.

**Table 2**
Association of log10 (CTRP7+CTRP15) levels with T2DM and IR in fully adjusted models.

| | T2DM | | | IR | | |
|---|---|---|---|---|---|---|
| Model adjust | OR | 95%CI | *p* | OR | 95%CI | *p* |
| Age, Sex | 1.048 | 1.035–1.061 | <0.001 | 1.026 | 1.020–1.032 | <0.001 |
| Age, Sex, BP, BMI | 1.049 | 1.036–1.063 | <0.001 | 1.025 | 1.019–1.031 | <0.001 |
| Age, Sex, BP, BMI, WC, Lipid profile | 1.062 | 1.034–1.090 | <0.001 | 1.021 | 1.014–1.027 | <0.001 |

Results of multivariable logistic regression analysis are presented 95%CI, confidence interval. OR is calculated by adding CTRP7 to CTRP15; BMI, body mass index; WC, waist circumference; BP, blood pressure; Lipid profile includes total cholesterol, triglyceride, low-density loprotein cholesterol, high-density lipoprotein cholesterol, and free fatty acid.

APN levels increased from level 2 to level 4 for T2DM (level 2: OR, 0.51; 95 % CI, 0.28–0.94; level 3: OR, 0.28; 95 % CI, 0.14–0.53; level 4: OR, 0.15; 95 % CI, 0.07–0.32) and for IR (level 2: OR, 0.37; 95 % CI, 0.20–0.70; level 3; OR, 0.26; 95 % CI, 0.13–0.50; level 4: OR, 0.17; 95 % CI, 0.08–0.34). Furthermore, using the Row Mean Score and Cochran-Armitage trend tests, we identified that the log10 (CTRP7+CTRP15) expression level was an independent risk factor for the development of T2DM and IR. The risk of T2DM and IR

**Table 3**
Row mean scores and Cochran–Armitage trend test of the impact of log10 (CTRP7+CTRP15) on T2DM and IR individuals.

| | T2DM | | IR | |
|---|---|---|---|---|
| | $\chi^2$ | *p* | $\chi^2$ | *p* |
| ROW Mean Scores Test | 106.1818 | <0.001 | 114.2183 | <0.001 |
| Cochran-Armitage Test | 98.1011 | <0.001 | 107.3213 | <0.001 |

increased with increasing log10 (CTRP7+CTRP15) (Table 3).

### 3.4. ROC curve analysis

To further evaluate the predictive value of log10 (CTRP7+CTRP15) levels for T2DM and IR incidence, we generated ROC curves and compared the area under the curve (AUC) among log10 (CTRP7+CTRP15), log10 (CTRP7), and log10 (CTRP15). The results showed that the AUC for log10 (CTRP7+CTRP15) in predicting the risk of T2DM was significantly higher than that of log10 (CTRP7) and log10 (CTRP15) ($AUC_{log10\ (CTRP7+CTRP15)}$: 0.93; $AUC_{log10\ (CTRP7)}$: 0.87, and $AUC_{log10\ (CTRP15)}$: 0.89) (Fig. 3E). Similar results were observed for IR ($AUC_{log10\ (CTRP7+CTRP15)}$: 0.86) (Fig. 3F). The sensitivity of $AUC_{log10\ (CTRP7+CTRP15)}$ for predicting T2DM was 83 %, with a specificity of 80 % and an optimal cut-off value of 2.32 (Fig. 3E), while the sensitivity for predicting IR was 82 %, with a specificity of 62 % and an optimal cut-off value of 2.33 (Fig. 3F).

### 3.5. Effects of multiple interventions on the serum CTRP7 and CTRP15 levels in healthy individuals

To further explore the regulatory factors of serum CTRP7 and CTRP15, we initially conducted an OGTT in healthy adults. As shown in Fig. 4A, the serum CTRP7 levels gradually decreased in the presence of high glucose and insulin levels, from 124.6 ± 9.9 μg/L to 99.5 ± 12.1 μg/L at 30 min and then to 94.05 ± 8.1 μg/L at 120 min. Serum CTRP15 also showed a declining trend under dual stimulation with high glucose and insulin, but the difference was not statistically significant (Fig. 4A).

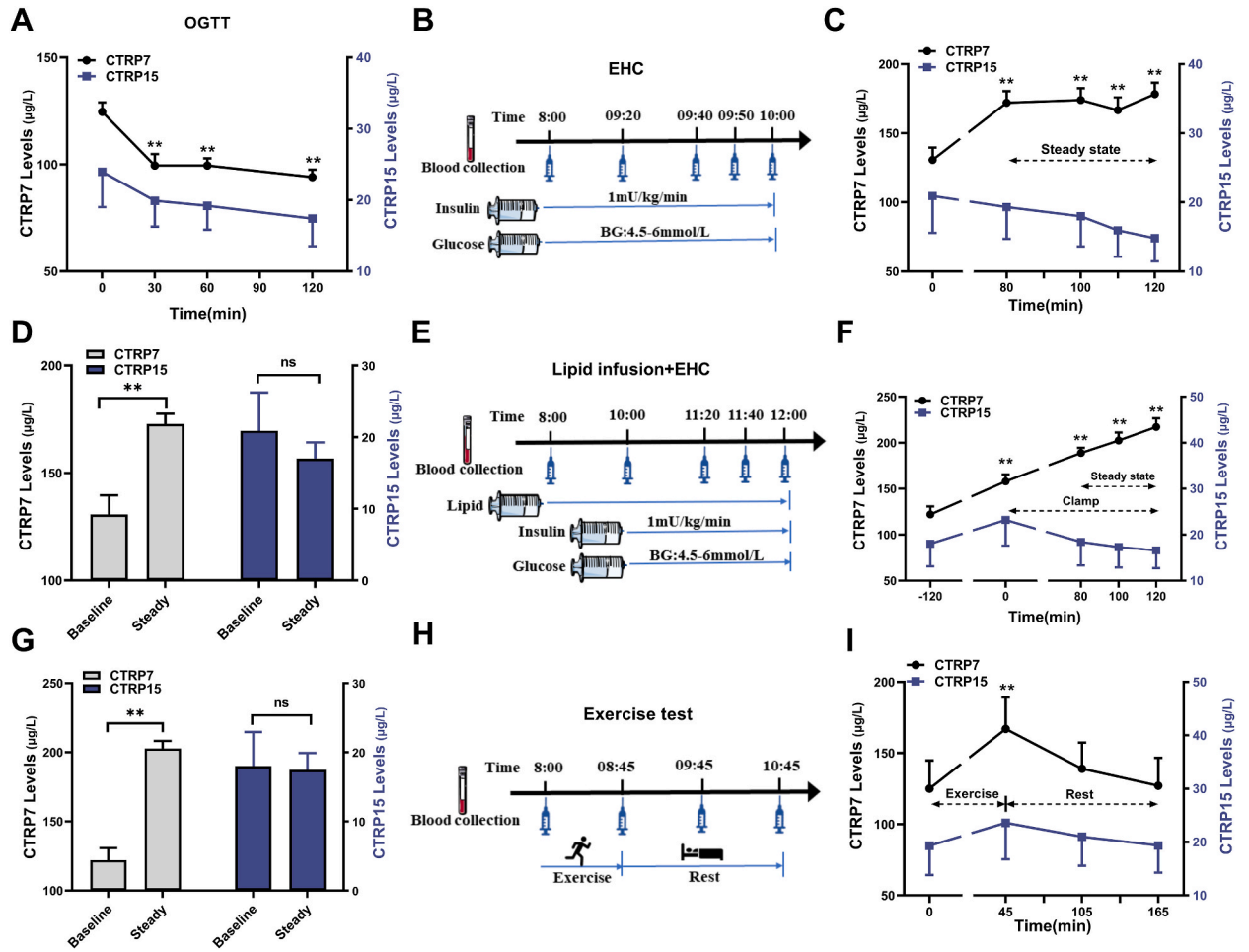


**Fig. 4.** Serum CTRP7 and CTRP15 levels in interventional studies. **(A)** Time course of changes in serum CTRP7 and CTRP15 levels in healthy subjects (n = 22) during the OGTT. **(B)** EHC protocol. **(C)** Time course of changes in serum CTRP7 and CTRP15 levels in healthy subjects (n = 24) during the EHC. **(D)** Serum CTRP7 and CTRP15 levels at baseline and the steady state of EHC. **(E)** Lipid infusion combined with the EHC protocol. **(F)** Changes in serum CTRP7 and CTRP15 levels in healthy subjects (n = 19) during lipid infusion combined with EHC. **(G)** Serum CTRP7 and CTRP15 levels at baseline and the steady state of lipid infusion combined with EHC. **(H)** Exercise test protocol. **(I)** Serum CTRP7 and CTRP15 levels in response to a 45 min bout of physical activity in healthy individuals. Data are means with 95 % CIs. $*p < 0.05$ or $**p < 0.01$ *vs.* control or baseline.

To further determine the effects of blood glucose and insulin levels on serum CTRP7 and CTRP15, we employed EHC to maintain blood glucose at baseline levels and increase circulating insulin levels (Fig. 4B). During the EHCs, blood glucose was clamped at 5–6 mmol/L, and insulin levels were increased from 43.0 ± 11.3 to 349.5 ± 53.4 pmol/L. Surprisingly, under high insulin levels, serum CTRP7 significantly increased (from 130.7 ± 21.1 μg/L to 172.8 ± 20.4 μg/L), while serum CTRP15 concentration did not significantly decrease (from 20.9 ± 12.6 μg/L to 17.01 ± 9.63 μg/L) (Fig. 4C and D). These results suggest that insulin promotes the secretion of serum CTRP7 but has a minimal effect on CTRP15.

Lipid infusion is known to elevate circulating FFA levels and induce acute IR [22]. To determine the relationships between serum CTRP7 and CTRP15 expression and IR, we performed a lipid infusion combined with an EHC experiment in healthy adults (Fig. 4E). As expected, 4 h of lipid infusion culminated in a pronounced decrease in the glucose infusion rate (GIR) and M value (from 10.12 ± 2.81 to 6.84 ± 2.06 mg/kg/min), indicating the occurrence of FFA-induced acute IR in *vivo*. As shown in Fig. 4F and G, lipid infusion-induced IR led to a significant increase in the serum CTRP7 concentration (from 122.1 ± 18.1 to 157.92 ± 15.8 μg/L, $p < 0.01$) compared with that at baseline. When the lipid infusion was complemented with EHC, there was an additional increase in the serum CTRP7 concentration (202.8 ± 20.2 μg/L, $p < 0.01$). However, the serum CTRP15 concentration exhibited an increasing trend after lipid infusion and then a decreasing trend during the clamp steady state, but these changes were not significant (Fig. 4F and G). Therefore, we believe that CTRP7 secretion increases in patients with high FFA and insulin levels, while CTRP15 levels remain relatively stable.

As the sole CTRP family member that originates from skeletal muscle, CTRP15 is hypothesized to be closely associated with physical activity and energy metabolism [23]. To test this hypothesis, we recruited healthy participants for a 45-min short-term cycling exercise experiment (Fig. 4H). Physical exercise notably stimulated the secretion of CTRP7, increasing from 125.0 ± 37.1 μg/L to

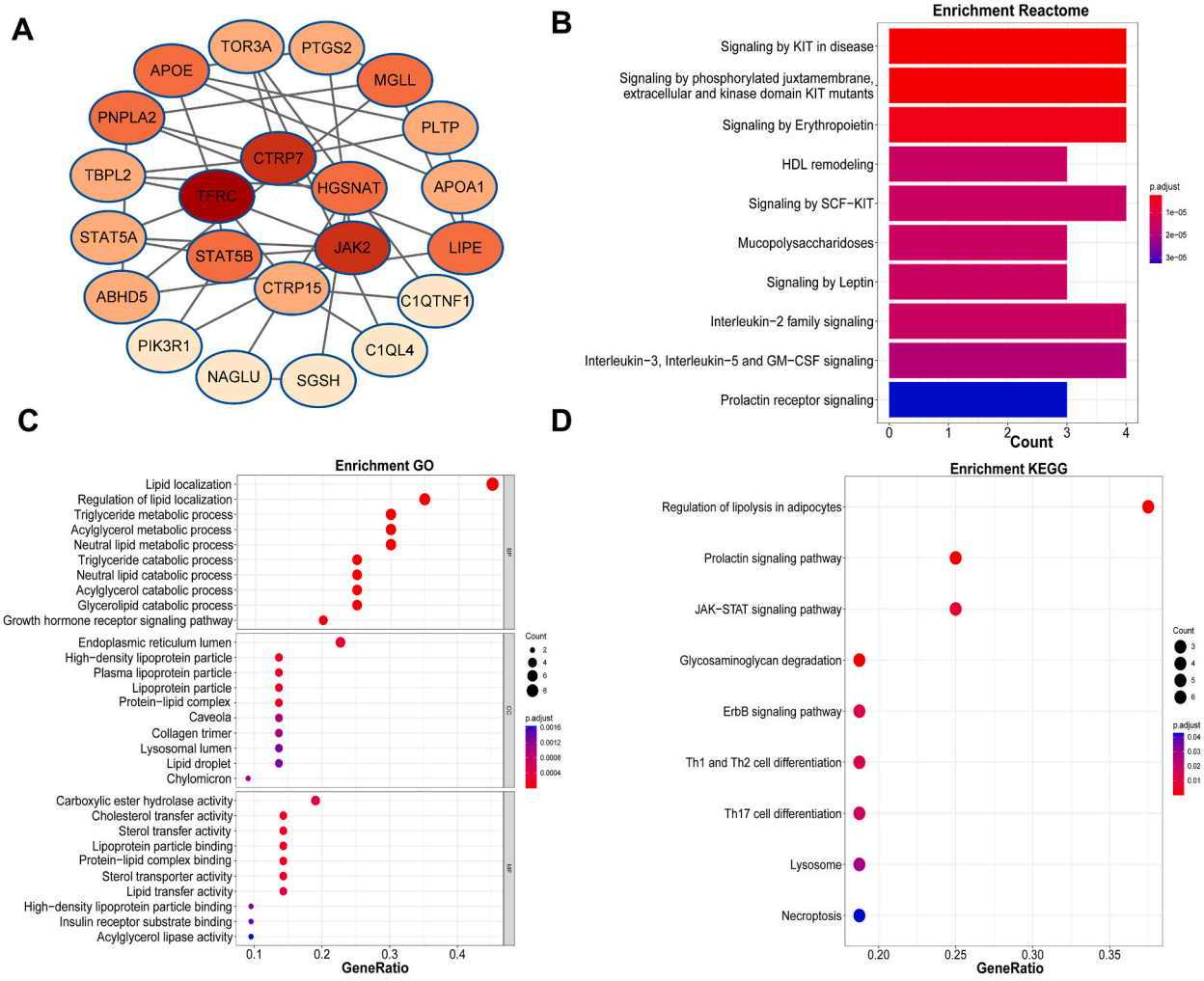


**Fig. 5.** Bioinformatics analysis related to CTRP7 and CTRP15. **(A)** The PPI network through the keywords CTRP7 and CTRP15 related to metabolism. **(B)** The enriched pathways of REACTOME. **(C)** and **(D)** The results of GO and KEGG analyses. The X-axis represents the ratio or count of involved genes, and the Y-axis represents GO, KEGG and REACTOME terms. The size of the bubbles indicates the number of genes involved, and each bubble represents a term. The darker the color, the smaller the *p* value.

170.0 ± 41.5 μg/L ($p < 0.01$) and returning to baseline levels after 75 min of rest. Intriguingly, CTRP15 expression exhibited an increasing trend postexercise compared with pre-exercise; however, the difference was not statistically significant (Fig. 4I). The results of physical exercise suggest that exercise may increase the secretion and release of CTRP7 in muscle tissue, but the effect of CTRP15 is not significant.

### 3.6. Bioinformatics analysis of CTRP7- and CTRP15-related genes and signaling pathways

#### 3.6.1. PPI network related to CTRP7 and CTRP15

We utilized the STRING database to predict proteins interacting with CTRP7 and CTRP15 and constructed a PPI network. Using REACTOME enrichment analysis, we identified metabolism-related pathways and genes enriched within these pathways. We ranked the genes based on their degree, from high to low, and chose the top 20 genes most closely related to CTRP7 and CTRP15 as core genes (TFRC, JAK2, STAT5B, HGSNAT, APOE, PNPLA2, MGLL, LIPE, STAT5A, TBPL2, TOR3A, PTGS2, PLTP, APOA1, ABHD5, PIK3R1, SGSH, NAGLU, C1QTNF1, C1QL4). We then concealed the remaining genes in the preliminary PPI network, ultimately obtaining a PPI network containing only CTRP7, CTRP15, and their core genes (Fig. 5A).

#### 3.6.2. REACTOME enrichment analysis

Based on the REACTOME enrichment analysis and using a $p$ value of $<0.05$ as the screening criterion, we ranked the values in descending order and found that CTRP7 and CTRP15 were primarily enriched in the following pathways: signaling by KIT in disease, signaling by phosphorylated juxtamembrane, signaling by erythropoietin, HDL remodelling, signaling by SCF-KIT, mucopolysaccharidoses, and signaling by leptin (Fig. 5B).

#### 3.6.3. GO analysis

According to the GO analysis results and using a $p$ value of $<0.05$ as the screening criterion, we ranked the values in descending order and selected the top 10 items in the biological process (BP), cellular component (CC), and molecular function (MF) categories. The results show that in BP, these proteins were primarily involved in lipid localization, regulation of lipid localization, triglyceride metabolic process, neutral lipid metabolic process, triglyceride catabolic process, and neutral lipid catabolic process. In CC, these proteins were enriched in endoplasmic reticulum (RE) lumen, high−density lipoprotein particle, plasma lipoprotein particle, lipoprotein particle, protein−lipid complex, and lipid droplet. In MF, the mentioned proteins were enriched in carboxylic ester hydrolase activity, cholesterol transfer activity, sterol transfer activity, lipoprotein particle binding, protein−lipid complex binding, and sterol transporter activity (Fig. 5C).

#### 3.6.4. KEGG analysis

Based on the KEGG analysis results, we used a $p$ value of $<0.05$ as the screening criterion and ranked the values in descending order. We found that CTRP7 and CTRP15 were mainly involved in the following processes: regulation of lipolysis in adipocytes, prolactin signaling pathway, AGE-RAGE signaling pathway in diabetic complications, growth hormone synthesis, secretion, and action, JAK-STAT signaling pathway, hepatitis B, glycosaminoglycan degradation, and cholesterol metabolism (Fig. 5D). Taken together, the bioinformatics analysis strongly suggested the involvement of CTRP7 and CTRP5 in metabolism-related physiological and pathological processes.

## 4. Discussion

In recent years, multiple studies have demonstrated the crucial role of CTRP family members, such as CTRP1, CTRP3, CTRP5, and CTRP6, in the development and progression of metabolic disorders related to obesity. They are implicated in the onset of diabetes and cardiovascular diseases by regulating insulin signaling, inflammatory pathways, and energy metabolism [5]. Our previous research also revealed that both serum CTRP7 and CTRP15 are related to the regulation of glucose/lipid metabolism and IR and may serve as serum biomarkers for metabolic-related diseases [7,8,12,13]. However, it remains unclear whether CTRP7 and CTRP15 have synergistic or antagonistic effects on IR. Our study revealed significantly elevated levels of CTRP7 and CTRP15 in both persons with IGT and T2DM, with higher levels observed in persons with T2DM. These findings suggested that circulating CTRP7 and CTRP15 levels are related to IR and increase further as IR progresses. Importantly, we found that log10 (CTRP7+CTRP15) also exhibited similar changes. Further analysis revealed that log10 (CTRP7+CTRP15) was significantly positively correlated with obesity- and IR-related indicators. Moreover, HOMA-IR, HbA1c, APN, BMI, and FFA were identified as independent factors affecting log10 (CTRP7+CTRP15). In contrast to APN levels, there was a notable association between higher log10 (CTRP7+CTRP15) levels and the risk of developing diabetes and IR. This compelling evidence signifies that log10 (CTRP7+CTRP15) reflects changes in metabolism and IR status and could potentially serve as a diagnostic marker for metabolic-related diseases.

It is well known that the concentrations of circulating cytokines, such as APN, zinc-a2-glycoprotein (ZAG), and secreted frizzled-related protein-5 (Sfrp5), are influenced by glucose, insulin, and metabolic disorders [24]. Therefore, we investigated how blood glucose, insulin, and FFA levels affect the levels of CTRP7 and CTRP15 in the blood. Through various intervention experiments, we found that during the OGTT, high glucose and high insulin concentrations led to a significant decrease in CTRP7, while the changes in CTRP15 were relatively small. When the blood glucose level in the EHC group was maintained at baseline, high insulin levels caused a significant increase in CTRP7 and a slight decrease in CTRP15. These results suggest that elevated insulin concentrations augment the secretion and release of CTRP7, while increased blood glucose levels hinder CTRP7 release. CTRP15 is mildly inhibited by blood

glucose and insulin. Therefore, it can be inferred that the regulatory activity of both cytokines is responsive to changes in blood glucose and insulin levels.

Investigations conducted on healthy cohorts have previously demonstrated that the administration of lipid/heparin infusion can elevate serum FFA concentrations, leading to acute IR and inhibiting whole-body glucose disposal [25,26] and insulin-dependent glucose uptake in forearm tissue during hyperglycemia and hyperinsulinemia [27]. To elucidate the correlation between serum CTRP7, CTRP15, and FFA levels, and acute IR, an experiment involving lipid infusion supplemented with EHC was conducted in healthy individuals. The results showed that high FFA levels led to a notable increase in CTRP7 levels, while CTRP15 levels slightly decreased, suggesting a mild dissociation of circulating CTRP7 and CTRP15 levels in acute IR states. These findings indicate that FFA significantly promotes CTRP7 release while mildly inhibiting CTRP15 secretion.

Skeletal muscle is considered an endocrine organ. Some cytokines, primarily secreted by adipose and muscle tissues, are referred to as adipo-myokines, and their levels, such as interleukin-6 (IL-6), IL-4, IL-7, and irisin are regulated by exercise [28–30]. To gain further insight into the interrelationship between CTRP7 and CTRP15 and muscle tissue, an experimental protocol involving 45 min of cycling exercise was administered to a group of healthy adults. The results revealed a substantial elevation in CTRP7 concentrations subsequent to the exercise regimen, whereas alterations in CTRP15 concentrations were minimal. These findings suggest that CTRP7 may function as a myokine whose expression is governed by physical exertion. However, further research should be conducted to determine the protein expression of CTRP7 and CTRP15 in muscle tissue before and after exercise.

To establish the groundwork for future investigations focusing on the interplay between CTRP7 and CTRP15 and between metabolic disorders and IR, we conducted a comprehensive bioinformatics analysis leveraging available on the Internet. We found that both factors were related to metabolism-associated genes and signaling pathways. In-depth research on CTRP7 and CTRP15 in relation to these genes and signaling pathways will contribute to advancements in IR research.

In summary, the objective of this study was to determine the intricate associations among CTRP7, CTRP15, metabolic disorders, and IR. Our findings revealed that the log10 (CTRP7+CTRP15) levels were notably elevated in persons with IGT and T2DM. The log10 (CTRP7+CTRP15) value was significantly associated with obesity, IR, and metabolic disorders, and its circulating levels were regulated by blood glucose and insulin. Further analysis suggests that log10 (CTRP7+CTRP15) may serve as a useful indicator for assessing IR and diagnosing T2DM, with potential clinical application value. However, our findings are applicable only to adult patients with diabetes or insulin resistance. We acknowledge that the limited sample size in this cross-sectional analytic study may introduce some constraints and potential biases to the results. Additionally, it remains unclear how serum levels of CTRP7 and CTRP15 vary with age. Therefore, future studies with larger sample sizes are warranted to validate our findings and provide a more comprehensive understanding. Additionally, future research should focus on the underlying mechanisms and potential therapeutic strategies targeting these cytokines to develop novel treatments for metabolic diseases.

## Ethics approval and consent to participate

This study was approved by the Human Research Ethics Committee of Chongqing Medical University (2012 Ethical Review No.74) and registered at the Chinese Clinical Trial Registry (ChiCTR–OCS–13003185). All participants provided informed consent to participate in the study.

## Data availability statement

Data will be made available on request.

## CRediT authorship contribution statement

**Shiyao Xue:** Writing – review & editing, Writing – original draft, Software, Formal analysis, Data curation, Conceptualization. **Jiaxiu Ling:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Mingyuan Tian:** Visualization, Validation, Investigation, Funding acquisition, Formal analysis. **Ke Li:** Software, Resources, Project administration, Methodology, Investigation. **Shengbing Li:** Validation, Supervision, Software, Resources, Formal analysis. **Dongfang Liu:** Validation, Supervision, Software, Formal analysis. **Ling Li:** Writing – review & editing, Visualization, Supervision, Software, Formal analysis, Data curation. **Mengliu Yang:** Writing – review & editing, Visualization, Validation, Methodology, Funding acquisition, Formal analysis. **Gangyi Yang:** Writing – review & editing, Visualization, Validation, Project administration, Methodology, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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